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# Differential effects of low- and high-intensity lower body negative pressure on noradrenaline and adrenaline kinetics in humans

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1. Lower body negative pressure provides a means to examine neurocirculatory reflexive responses to decreases in venous return to the heart. We assessed whether the pattern of catecholaminergic responses to lower body negative pressure depends on the intensity of the stimulus (–15 versus –40 mmHg).

2. In 14 healthy subjects, responses of forearm blood flow and noradrenaline spillover and of total body noradrenaline and adrenaline spillover were assessed during infusion of [<sup>3</sup>H]noradrenaline and [<sup>3</sup>H]adrenaline during –15 and –40 mmHg of lower body negative pressure.

3. During lower body negative pressure at –15 mmHg, heart rate and pulse pressure did not change, but forearm vascular resistance increased by 25–50%. Forearm noradrenaline spillover increased by about 50%, from  $0.63 \pm 0.16$  to  $0.94 \pm 0.23$  pmol min<sup>–1</sup> 100 ml<sup>–1</sup> ( $P < 0.05$ ). Total body noradrenaline spillover did not change, and total body adrenaline spillover increased significantly by about 30%. Clearances of noradrenaline and adrenaline were unchanged.

4. During lower body negative pressure at –40 mmHg, heart rate increased and pulse pressure decreased. Forearm vascular resistance increased by about 100%, and forearm noradrenaline spillover increased by 80%, from  $0.73 \pm 0.19$  to  $1.32 \pm 0.36$  pmol min<sup>–1</sup> 100 ml<sup>–1</sup> ( $P < 0.05$ ). Total body noradrenaline spillover increased by 30%, and total body adrenaline spillover increased by about 50%. Clearances of both noradrenaline and adrenaline decreased.

5. The results are consistent with the view that selective deactivation of cardiopulmonary baroreceptors during low-intensity lower body negative pressure increases sympathoneural traffic to forearm skeletal muscle and increases adrenomedullary secretion with-

out a concomitant generalized increase in sympathoneural outflows. Concurrent deactivation of cardiopulmonary and arterial baroreceptors during high-intensity lower body negative pressure evokes a more generalized increase in sympathoneural activity, accompanied by further increased adrenomedullary secretion and decreased plasma clearances of noradrenaline and adrenaline. The findings support differential increases in skeletal sympathoneural and adrenomedullary outflows during orthostasis, with more generalized sympathoneural responses to systemic hypotension.

## INTRODUCTION

The lower body negative pressure (LBNP) technique, introduced by Greenfield et al. [1] in 1963, has been used frequently for examination of cardiopulmonary baroreflex function. By applying different levels of subatmospheric pressure to the lower half of the body, one can study reflexive neurocirculatory responses to decreases in venous return to the heart. Results of the studies of Zoller et al. [2] and Johnson et al. [3] have led to the view that LBNP at intensities less than –20 mmHg selectively deactivates cardiopulmonary baroreceptors and that, in humans, alterations in cardiopulmonary baroreceptor afferent traffic preferentially influence sympathetic outflows to skeletal muscle and skin. LBNP at intensities greater than –20 mmHg also deactivates arterial baroreceptors. Microneurographic studies have shown that both low- and high-intensity LBNPs increase efferent sympathoneural outflows to skeletal muscles in the arms and legs [4–6]. Because of differentiated autonomic response patterns during different forms of stress, microneuro-

**Key words:** adrenaline, baroreceptors, cardiopulmonary, lower body negative pressure, noradrenaline.

**Abbreviations:** AD, adrenaline; CL, clearance; FBF, forearm blood flow; FPF, forearm plasma flow; FVR, forearm vascular resistance; LBNP, lower body negative pressure; NA, noradrenaline.

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graphic data about skeletal muscle and skin sympathetic nerve activity may not detect alterations in sympathetic nerve activity in other organs.

Using a tracer approach, Baily et al. [7] reported increases in forearm noradrenaline (NA) spillover but not in 'total' body NA spillover (NA entry into arterial plasma) during low-intensity LBNP. Total body NA spillover responses to high-intensity LBNP have not been reported. Little is known about baroreflex regulation of adrenomedullary secretion in humans during LBNP or orthostasis. One study reported an increase in venous plasma adrenaline (AD) levels during low-intensity LBNP in humans [8]. The meaning of this finding is unclear, since venous plasma AD levels depend not only on adrenomedullary secretion but also on clearance of AD from arterial plasma and on local catecholamine removal in the forearm.

This study assessed the effects of low- and high-intensity LBNP on total body and forearm kinetics of NA and AD in healthy humans, using steady-state infusions of tracer amounts of [ $^3\text{H}$ ]NA and [ $^3\text{H}$ ]AD [9].

## MATERIALS AND METHODS

### Subjects

Fourteen healthy male subjects (aged 25–38 years) participated in the study after giving their written informed consent. The study protocol was approved by the hospital ethics committee. Before entry into the study, all participants had a normal physical examination. None suffered from cardiovascular or other major diseases, and they took no medication.

### Study protocol

All subjects abstained from nicotine, alcohol, and caffeinated foods and beverages for at least 24 h before the study. The subjects were allowed to eat a light breakfast 2 h before the study. All experiments were carried out in the morning in a temperature-controlled observation room.

During the study the subjects remained supine, with the lower body sealed at the iliac crests in an airtight Plexiglass<sup>TM</sup> box. The LBNP applied was recorded by a manometer connected to the inside of the box.

After local anaesthesia using lidocaine, a cannula was inserted percutaneously into a brachial artery, for monitoring arterial blood pressure and heart rate (Hewlett Packard, Boblingen, Germany) and for drawing arterial blood samples. In the same arm, a cannula was inserted for collecting venous blood. Another venous cannula was inserted in the contralateral arm, for infusion of [ $^3\text{H}$ ]NA or [ $^3\text{H}$ ]AD.

Forearm blood flow (FBF) was measured using venous occlusion strain-gauge plethysmography [10] on the forearm contralateral to that used for

[ $^3\text{H}$ ]NA infusion and it was positioned 10 cm above the mid-thoracic level. Measurements of FBF were obtained after exclusion of the hand circulation by inflating a wrist cuff to 100 mmHg above systolic blood pressure for at least 1 min [11].

After instrumentation, the subjects rested for 30 min. During the last 3 min, baseline recordings of blood pressure, heart rate and FBF (nine flow curves) were obtained. Blood pressure was recorded simultaneously with each FBF measurement. Thereafter, arterial and venous blood samples were drawn simultaneously for determinations of plasma catecholamines. Six subjects were infused with the radiotracer [ $^3\text{H}$ ]NA (L-2,5,6-[ $^3\text{H}$ ]NA) (specific activity 30–60 Ci/mmol) and another six were infused with [ $^3\text{H}$ ]AD (L-N-methyl-[ $^3\text{H}$ ]AD) (specific activity 55–85 Ci/mmol). An intravenous bolus of  $15 \mu\text{Ci}/\text{m}^2$  ( $0.55 \text{ MBq}/\text{m}^2$ ) was administered, followed by a constant infusion of  $0.35 \mu\text{Ci}/\text{min}$  ( $0.013 \text{ MBq}/\text{min}$ ) for 120 min. In two subjects, [ $^3\text{H}$ ]NA and [ $^3\text{H}$ ]AD were infused simultaneously. The first LBNP application began 30 min after the start of infusion of the radiotracers.

LBNP at  $-15 \text{ mmHg}$  was applied for 30 min. Blood pressure, heart rate, FBF and blood samples were taken in sequence, beginning after 7, 17 and 27 min of LBNP, so that the blood samples were drawn at 10, 20 and 30 min of LBNP. A 30-min rest period ensued, followed by another 30 min of LBNP at  $-40 \text{ mmHg}$ . Blood pressure, pulse rate, FBF and blood samples were obtained at the same time points as during LBNP at  $-15 \text{ mmHg}$ .

### Materials

Tritiated NA and AD were obtained from Du Pont New England Nuclear ('s-Hertogenbosch, The Netherlands). The radionuclides were sterilized using a  $0.22 \mu\text{m}$  filter and diluted in 0.9% NaCl containing acetic (0.2 mol/l) and ascorbic (1 mg/ml) acids. Aliquots of  $70 \mu\text{Ci}$  of [ $^3\text{H}$ ]NA and [ $^3\text{H}$ ]AD were stored at  $-80^\circ\text{C}$  until used. Sterilization, dilution and aliquoting were carried out under nitrogen. Just before use, an aliquot was diluted in 0.9% NaCl. The syringe containing the radiotracer was weighed just before and just after the infusion, in order to verify the infusion rate. Samples of the infusates were taken at the end of the infusion and stored at  $-80^\circ\text{C}$ .

### Analytical methods

Blood samples were collected in chilled tubes containing glutathione (0.2 mol/l) and EGTA (0.25 mol/l). The tubes were centrifuged at  $4^\circ\text{C}$  and the plasma was separated and stored at  $-20^\circ\text{C}$ . Assays of samples and infusates were carried out within 2 months of each study.

Plasma samples were assayed for concentrations of AD and NA using HPLC with fluorimetric



**Table 1. Haemodynamic variables before (basal) and during LBNP at -15 and -40 mm Hg.** Values are expressed as means  $\pm$  SEM. Statistical significance (Wilcoxon signed-rank test): \* $P < 0.05$ .

	Basal	LBNP (-15 mmHg)	Basal	LBNP (-40 mmHg)
<b>[<sup>3</sup>H]NA infusion (n = 8)</b>				
Mean arterial pressure (mmHg)	90 $\pm$ 3	89 $\pm$ 3	92 $\pm$ 2	93 $\pm$ 3
Pulse pressure (mmHg)	66 $\pm$ 3	63 $\pm$ 4	62 $\pm$ 3	55 $\pm$ 3*
Heart rate (beats/min)	58 $\pm$ 4	57 $\pm$ 4	56 $\pm$ 4	63 $\pm$ 4*
FBF (ml min <sup>-1</sup> 100 ml <sup>-1</sup> )	1.81 $\pm$ 0.27	1.38 $\pm$ 0.22*	1.85 $\pm$ 0.24	1.00 $\pm$ 0.22*
FVR (arbitrary units)	64 $\pm$ 3	80 $\pm$ 12*	57 $\pm$ 8	122 $\pm$ 19*
<b>[<sup>3</sup>H]AD infusion (n = 8)</b>				
Mean arterial pressure (mmHg)	81 $\pm$ 2	83 $\pm$ 2	88 $\pm$ 2	90 $\pm$ 2
Pulse pressure (mmHg)	58 $\pm$ 3	57 $\pm$ 3	53 $\pm$ 5	47 $\pm$ 6*
Heart rate (beats/min)	54 $\pm$ 3	53 $\pm$ 2	53 $\pm$ 2	61 $\pm$ 3*
FBF (ml min <sup>-1</sup> 100 ml <sup>-1</sup> )	1.59 $\pm$ 0.23	1.11 $\pm$ 0.20*	1.49 $\pm$ 0.25	0.90 $\pm$ 0.23*
FVR (arbitrary units)	59 $\pm$ 8	90 $\pm$ 14*	74 $\pm$ 12	147 $\pm$ 32*

detection after precolumn derivatization with the fluorescent agent 1,2-diphenylethylenediamine [12]. A Gilson fraction collector (Model 201-202), which was connected to an automatic sample injector (Wisp 710B), was used for collecting [<sup>3</sup>H]NA and [<sup>3</sup>H]AD into scintillation vials, according to the retention times of NA and AD standards.

### Data analysis

Forearm vascular resistance (FVR) was calculated by dividing mean arterial blood pressure by FBF and was expressed in resistance units.

The clearance of NA from arterial plasma, CL, was calculated from the infusion rate of [<sup>3</sup>H]NA and the steady-state arterial plasma concentration of the tracer, [<sup>3</sup>H]NA<sub>a</sub>:

$$CL \text{ (l/min)} = \text{infusion rate (d.p.m./min)} / [\text{d.p.m./l}] [\text{d.p.m./l}]$$

'Total body' NA spillover, the estimated rate of appearance of endogenous NA in arterial plasma, representing generalized sympathetic nerve activity, was calculated from the arterial plasma NA concentration (NA<sub>a</sub>) and the arterial steady-state clearance of NA, according to the equation:

$$\text{Total body NA spillover (nmol/min)} = NA_a \text{ (nmol/l)} \times CL \text{ (l/min)}$$

Analogously, NA spillover in the forearm was estimated from:

$$\text{Forearm spillover (pmol min}^{-1} \text{ 100 ml}^{-1}) = [FPF \times NA_a \times f_{NA}] + [FPF \times (NA_v - NA_a)]$$

where  $f_{NA} = ([\text{d.p.m.}] NA_a - [\text{d.p.m.}] NA_v) / [\text{d.p.m.}] NA_a$ , the fractional extraction of the tracer in the forearm; FPF is the forearm plasma flow, in units of ml min<sup>-1</sup> 100 ml<sup>-1</sup>, calculated from the FBF and haematocrit; and [<sup>3</sup>H]NA<sub>v</sub> is the venous plasma concentration of [<sup>3</sup>H]NA.

The removal of NA in the forearm was calculated from:

$$\text{Forearm removal (pmol min}^{-1} \text{ 100 ml}^{-1}) = FPF \times NA_a \times f_{NA}$$

The clearance of AD from arterial plasma and the estimated rate of appearance of endogenous AD in arterial plasma were calculated according to the same formulas.

Results are expressed as means  $\pm$  SEM. For each variable, the mean of the three observations at the three time points during each LBNP was calculated. Within each group, the effects of LBNP on each variable were tested by the Wilcoxon signed-rank test. This test was also used for testing differences between LBNP at -15 and at -40 mmHg. A *P*-value below 0.05 defined statistical significance.

## RESULTS

### Baseline measurements.

At baseline, total body spillover of NA (2.81  $\pm$  0.34 nmol/min) was about seven to eight times higher than that of AD (0.36  $\pm$  0.05 nmol/min). Clearances of NA and AD from arterial plasma were similar (3.17  $\pm$  0.34 and 2.84  $\pm$  0.14 l/min).

### LBNP at -15 mmHg

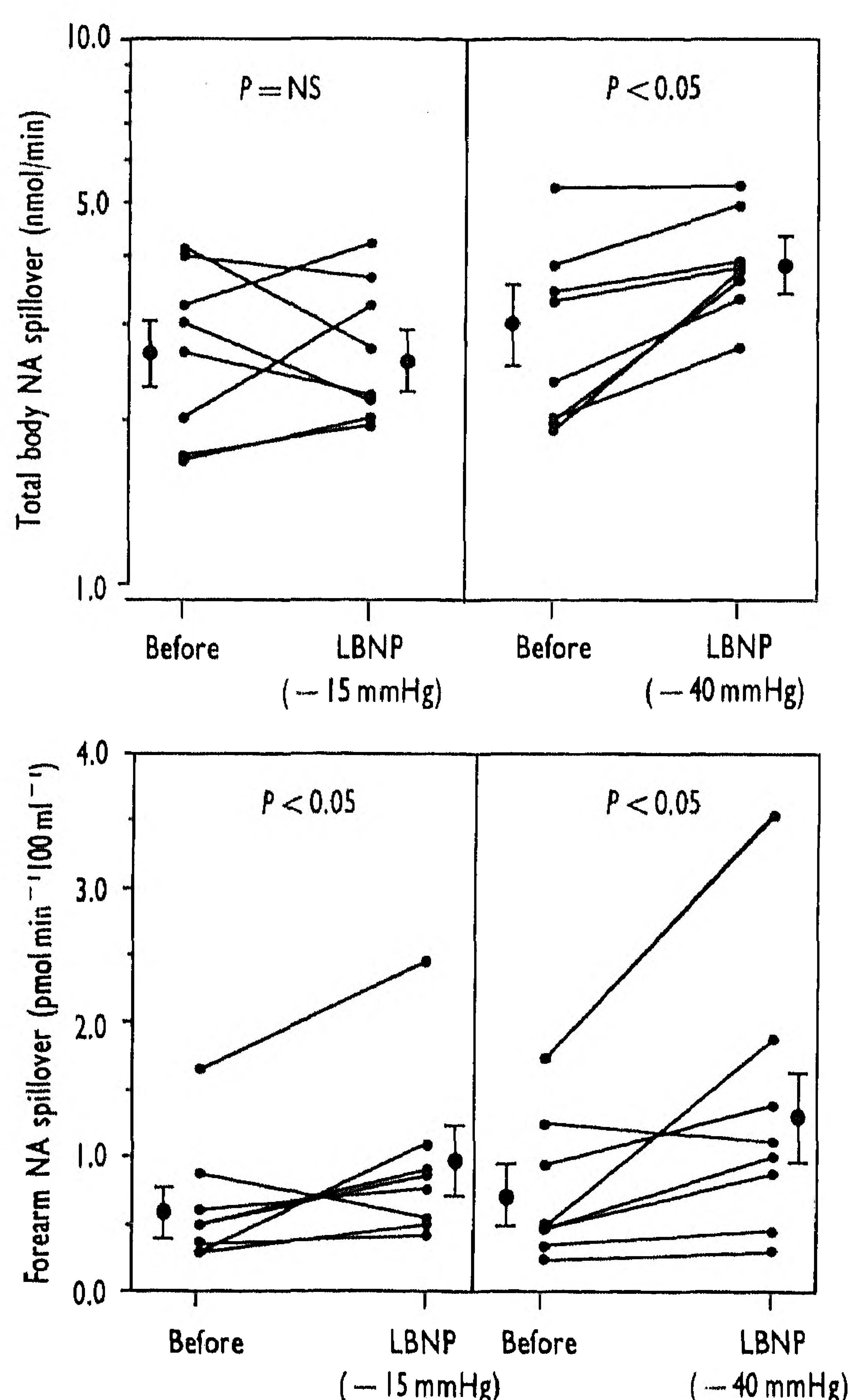
Mean arterial blood pressure, pulse pressure and heart rate did not change during LBNP at -15 mmHg (Table 1). FBF decreased significantly by about 30% and FVR increased by about 25–50%.

The increase in venous plasma NA was larger than that of arterial plasma NA during LBNP at -15 mmHg, while the increase in venous plasma AD was smaller than that of arterial plasma AD (Table 2). The total body spillover of NA did not increase during LBNP at -15 mmHg (Fig. 1), whereas the forearm spillover of NA increased in seven out of eight subjects, from 0.63  $\pm$  0.16 to 0.94  $\pm$  0.23 pmol min<sup>-1</sup> 100 ml<sup>-1</sup> ( $P < 0.05$ ) (Fig. 1). The clearance of NA from arterial plasma tended to decrease ( $P < 0.10$ ). The removal of NA from the forearm did not change during LBNP at

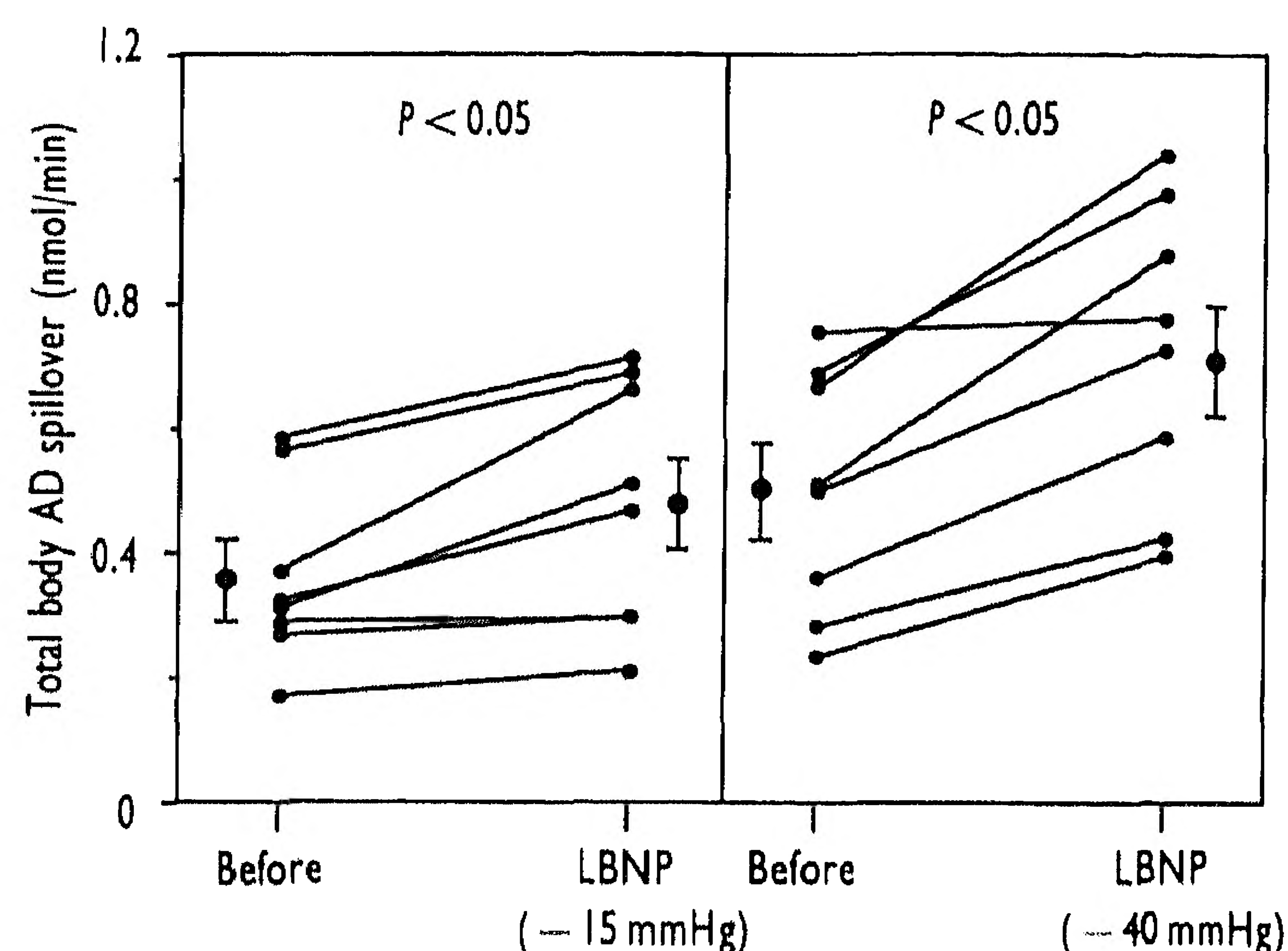


**Table 2. Plasma concentrations of NA and AD, total body and forearm spillover and removal of NA before (basal) and during LBNP at -15 and -40 mmHg.** Values are expressed as means  $\pm$  SEM. Statistical significance (Wilcoxon signed-rank test): \* $P < 0.05$ .

	Basal	LBNP (-15 mmHg)	Basal	LBNP (-40 mmHg)
<b>[<sup>3</sup>H]NA infusion (n = 8)</b>				
Venous NA (nmol/l)	0.90 $\pm$ 0.10	1.44 $\pm$ 0.20*	1.13 $\pm$ 0.19	2.60 $\pm$ 0.30*
Arterial NA (nmol/l)	0.90 $\pm$ 0.06	1.10 $\pm$ 0.10*	1.01 $\pm$ 0.12	1.88 $\pm$ 0.07*
Total body NA spillover (nmol/min)	2.81 $\pm$ 0.34	2.78 $\pm$ 0.30	3.00 $\pm$ 0.42	3.89 $\pm$ 0.30*
NA clearance (l/min)	3.17 $\pm$ 0.34	2.57 $\pm$ 0.19	3.12 $\pm$ 0.39	2.08 $\pm$ 0.15*
Forearm NA spillover (pmol min <sup>-1</sup> 100 ml <sup>-1</sup> )	0.63 $\pm$ 0.16	0.94 $\pm$ 0.23*	0.73 $\pm$ 0.19	1.32 $\pm$ 0.36*
Forearm NA removal (pmol min <sup>-1</sup> 100 ml <sup>-1</sup> )	0.62 $\pm$ 0.11	0.67 $\pm$ 0.13	0.70 $\pm$ 0.15	0.91 $\pm$ 0.24
<b>[<sup>3</sup>H]adrenaline infusion (n = 8)</b>				
Venous AD (nmol/l)	0.06 $\pm$ 0.01	0.08 $\pm$ 0.01*	0.07 $\pm$ 0.01	0.14 $\pm$ 0.02*
Arterial AD (nmol/l)	0.13 $\pm$ 0.02	0.18 $\pm$ 0.02*	0.19 $\pm$ 0.02	0.34 $\pm$ 0.03*
Total body AD spillover (nmol/min)	0.36 $\pm$ 0.05	0.48 $\pm$ 0.07*	0.50 $\pm$ 0.07	0.72 $\pm$ 0.08*
AD clearance (l/min)	2.84 $\pm$ 0.14	2.63 $\pm$ 0.17	2.51 $\pm$ 0.18	2.07 $\pm$ 0.16*
Forearm AD removal (pmol min <sup>-1</sup> 100 ml <sup>-1</sup> )	0.07 $\pm$ 0.01	0.07 $\pm$ 0.01	0.10 $\pm$ 0.02	0.11 $\pm$ 0.03



**Fig. 1. Total body NA spillover and forearm NA spillover before and in response to LBNP at -15 and -40 mmHg.** NS, not significant.



**Fig. 2. Total body AD spillover before and in response to LBNP at -15 and -40 mmHg.**

-15 mmHg (Table 2). The fractional extraction of NA increased slightly from  $0.64 \pm 0.04$  to  $0.73 \pm 0.03$  ( $P < 0.05$ ).

The total body spillover of AD increased by about 30% during LBNP at -15 mmHg, from  $0.36 \pm 0.05$  to  $0.48 \pm 0.07$  nmol/min (Table 2). The individual responses of total body spillover of AD are shown in Fig. 2. The clearance of AD from arterial plasma and the removal of AD from the forearm did not change during LBNP at -15 mmHg (Table 2).

#### **LBNP at -40 mmHg**

Mean arterial blood pressure did not change significantly during LBNP at -40 mmHg but pulse pressure decreased and heart rate increased signifi-



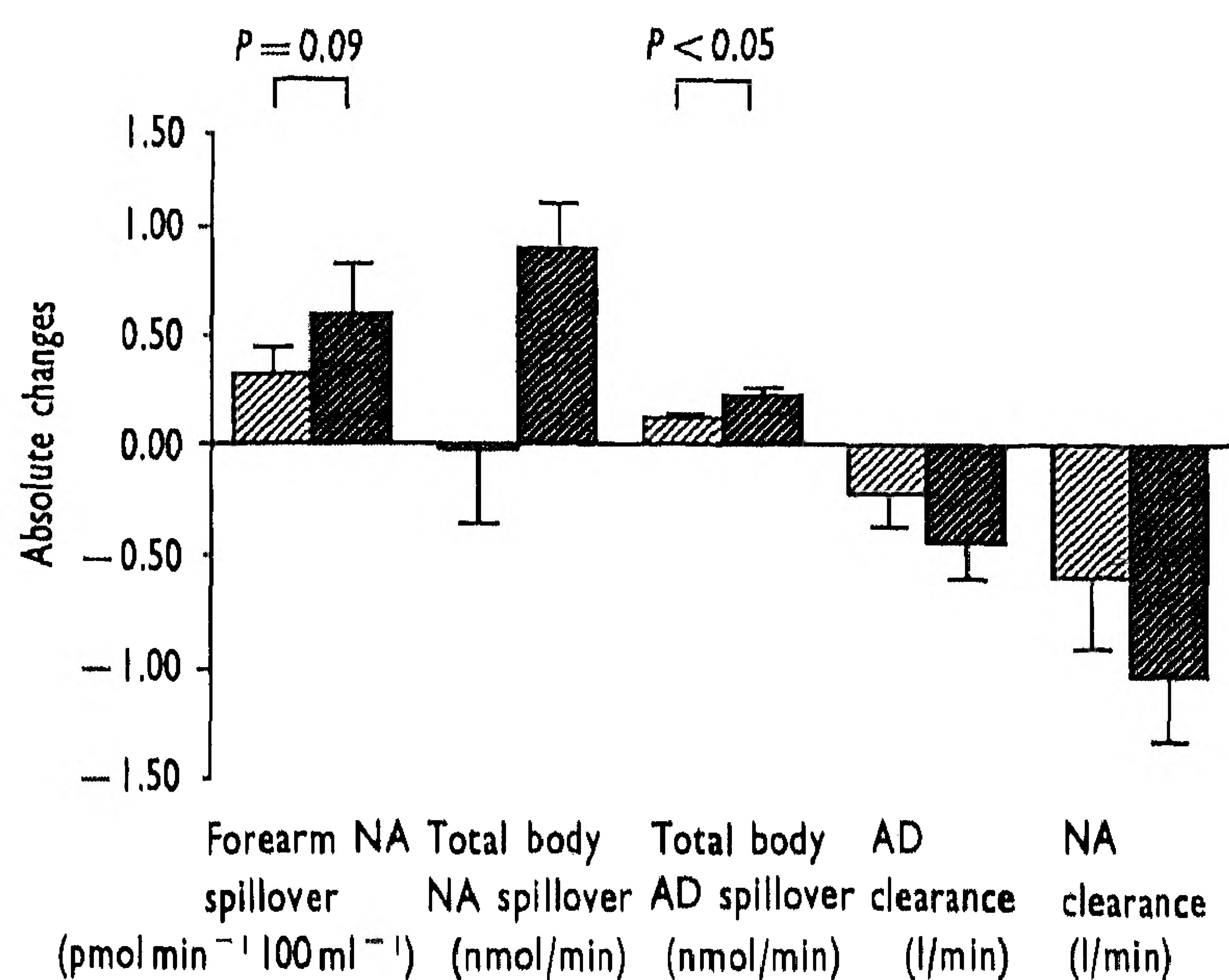


Fig. 3. Absolute responses of forearm NA spillover, total body NA spillover and NA clearance in eight subjects, and of total body AD spillover and AD clearance in eight subjects, to LBNPs at  $-15$  (▨) and  $-40$  mmHg (■)

cantly in both groups (Table 1). FBF decreased significantly by about 40–50% and the FVR increased by about 100%.

The increase in venous plasma NA was larger than that of arterial plasma NA during LBNP at  $-40$  mmHg (Table 2). In contrast to LBNP at  $-15$  mmHg, the total body spillover of NA increased by about 30% during LBNP at  $-40$  mmHg, while the forearm spillover of NA increased by about 80% from  $0.73 \pm 0.19$  to  $1.32 \pm 0.36$  pmol min<sup>-1</sup> 100 ml<sup>-1</sup> (Fig. 1). Figure 1 shows that the forearm spillover of NA increased in seven out of eight subjects. The clearance of NA from arterial plasma decreased significantly while the removal of NA from the forearm did not change significantly during LBNP at  $-40$  mmHg (Table 2). The fractional extraction of NA increased slightly from  $0.62 \pm 0.05$  to  $0.76 \pm 0.03$  ( $P < 0.05$ ) during LBNP at  $-40$  mmHg.

The total body spillover of AD increased by about 50% in all subjects during LBNP at  $-40$  mmHg (Table 2 and Fig. 2). The clearance of AD from arterial plasma decreased while removal of AD from the forearm remained unchanged (Table 2).

#### Comparison of LBNP at $-15$ and $-40$ mmHg

Comparison of the responses during LBNP at  $-15$  and  $-40$  mmHg was made for the variables whose values changed during both LBNP periods. The increase in FVR was larger during LBNP at  $-40$  than at  $-15$  mmHg ( $P < 0.05$ ) (Table 1). The increments in venous and arterial plasma NA and AD were also significantly larger during LBNP at  $-40$  than at  $-15$  mmHg (Table 2). The increase in forearm spillover of NA tended to be larger at  $-40$  mmHg ( $0.59 \pm 0.24$  pmol min<sup>-1</sup> 100 ml<sup>-1</sup>) than at  $-15$  mmHg ( $0.31 \pm 0.13$  pmol min<sup>-1</sup> 100 ml<sup>-1</sup>;  $P = 0.09$ , Fig. 3). The increment in total body spil-

lover of AD was larger during LBNP at  $-40$  mmHg ( $0.22 \pm 0.04$  nmol/min) than at  $-15$  mmHg ( $0.12 \pm 0.03$  nmol/min;  $P < 0.05$ , Fig. 3).

#### DISCUSSION

The main new findings of this study were: (i) low-intensity LBNP increased forearm NA spillover and total body AD spillover without affecting total body NA spillover; (ii) high-intensity LBNP increased total body NA spillover; (iii) LBNP elicited intensity-related increments in total body spillover of AD and decreases in plasma clearances of NA and AD.

Zoller et al. [2] and Johnson et al. [3] demonstrated that a LBNP of less than  $-20$  mmHg selectively deactivates cardiopulmonary baroreceptors. At negative pressures exceeding  $-20$  mmHg, there is additional deactivation of arterial baroreceptors. Our data are in agreement with selective deactivation of cardiopulmonary baroreceptors during low-intensity LBNP ( $-15$  mmHg), since we found no increase in heart rate or a decrease in pulse pressure in response to this stimulus. During LBNP at  $-40$  mmHg, heart rate increased and pulse pressure decreased. At this stage, it is impossible to examine arterial baroreceptors separately from cardiopulmonary baroreceptors with LBNP since arterial baroreceptor deactivation cannot be attained without simultaneous perturbation of the cardiopulmonary baroreceptors. Two other factors impede an examination of the arterial baroreceptors separately. First, the stronger intensity of LBNP at  $-40$  mmHg does not only cause a concurrent deactivation of arterial baroreceptors but probably also elicits a gradually stronger deactivation of cardiopulmonary baroreceptors. Second, there is a functional interaction between arterial and cardiopulmonary baroreceptors that does not allow assessment of effects of arterial baroreceptor deactivation by subtracting the effects of unloading of cardiopulmonary baroreceptors from that of simultaneous deactivation of arterial and cardiopulmonary baroreceptors.

During low-intensity LBNP, there was an increase in forearm NA spillover but not in total body NA spillover. This not only confirms that cardiopulmonary baroreceptors play an important role in reflexive regulation of FBF but also indicates the regionalization of sympathoneuronal responses to this stressor. In contrast, total body spillover of NA increased during concomitant deactivation of cardiopulmonary and arterial baroreceptors, suggesting more diffuse increases in sympathetic nerve outflows. Measurements of total body spillover of NA during orthostasis may therefore fail to detect regional sympathoneuronal responses. Differentiated sympathoneuronal response patterns have also been demonstrated for several other stressors.



The plasma clearance of NA tended to decrease during cardiopulmonary baroreceptor deactivation and decreased further during simultaneous deactivation of arterial and cardiopulmonary baroreceptors. These decreases in plasma NA clearance probably resulted from decreases in cardiac output and splanchnic blood flow, as has been demonstrated during cardiopulmonary and arterial baroreceptor deactivation [13, 14].

Apart from sympathoneural activation during LBNP, we also found small but significant elevations in venous and arterial AD levels. The increments were larger during high-intensity LBNP than during low-intensity LBNP. Increments in plasma AD levels during low-intensity LBNP are in agreement with a previous study [8]. Since we found no decrease in the clearance of AD and an increase in total body spillover of AD, the increments in plasma AD levels during low-intensity LBNP probably result from increased adrenomedullary secretion. During combined deactivation of cardiopulmonary and arterial baroreceptors, there was a further increase in adrenomedullary secretion of AD, but now the decreased clearance of AD contributed to the larger increment in plasma AD levels during high-intensity LBNP.

LBNP at  $-45$  to  $-50$  mmHg is equivalent to head-up tilt to  $90^\circ$ , with respect to the amount of blood translocated. As shown previously, plasma NA and AD levels nearly double during orthostasis, and this is in agreement with the plasma catecholamine responses during LBNP at  $-40$  mmHg in the present study. These increments in plasma catecholamine levels result from both increased arterial NA spillover and decreased plasma NA clearance [15]. In the present study, using LBNP at  $-40$  mmHg, responses of spillover and clearance were qualitatively similar to those reported previously for head-up tilt.

LBNP induced a vasoconstrictor response in the forearm that was largest when both types of baroreceptors were deactivated simultaneously during high-intensity LBNP. This is in agreement with the further increase in forearm NA spillover during high-intensity LBNP. The larger responses of FVR and forearm NA spillover during high-intensity LBNP can be explained by either a stronger deactivation of cardiopulmonary baroreceptors, or by the concurrent deactivation of arterial baroreceptors or both. Since there must be arterial baroreceptor deactivation during high-intensity LBNP [2, 3], our data indicate that arterial baroreceptors are probably as important for baroreflex control of the forearm skeletal muscle circulation as cardiopulmonary baroreceptors. This conclusion is supported by the findings of microneurographic studies, showing that sympathetic nerve traffic responses were larger during deactivation of arterial baroreceptors than during deactivation of cardiopulmonary baroreceptors [6, 16]. Previous studies have suggested that cardiopulmonary baroreceptors play a major role in

the reflex control of the skeletal muscle circulation [17].

The contribution of the skin to the FBF and thus to the forearm NA spillover should be considered. Some but not all previous studies showed that LBNP produced graded vasoconstriction of the skin vessels, thus contributing to the forearm vasoconstrictor response [7, 18]. Assuming that sympathetic nerve traffic to the skin is also increased during LBNP, the increase in forearm NA spillover may overestimate the increase in muscle sympathoneural activity; however, if the increments in NA spillover from the skin and the muscles were proportionally the same at  $-15$  and at  $-40$  mmHg, then the conclusion that arterial baroreceptors are important for reflex regulation of skeletal muscle circulation would be still valid.

In conclusion, the data of this study indicate that selective deactivation of cardiopulmonary baroreceptors during low-intensity LBNP increases NA release in the forearm and AD release by the adrenals. Concurrent deactivation of arterial baroreceptors during high-intensity LBNP produces further increases in forearm vasoconstriction and NA spillover, with increases also in total body NA and AD spillover and decreases in clearances of NA and AD.

## ACKNOWLEDGMENT

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